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RESEARCH ARTICLE

Germination ecology of the critically endangered sainfoin *Onobrychis conferta* subsp. *conferta* and the rare *O. caput-galli* (Fabaceae)

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Abstract

Background and aims – Understanding germination and seedling emergence responses of pastoral legumes is essential for improving conservation and restoration efforts, particularly in arid and semi-arid ecosystems. This study aimed to characterize the germination ecology of two threatened taxa, *Onobrychis conferta* subsp. *conferta* and *Onobrychis caput-galli*, which have potential value for rangeland restoration and forage production, to assess their tolerance to key environmental factors.

Material and methods – Germination and seedling emergence were investigated under controlled laboratory and greenhouse conditions. Seeds were exposed to a range of temperatures at four alternating day/night temperatures (15/5, 20/10, 25/15, and 30/20°C) and four constant temperatures (17, 21, 25, and 29°C), light conditions were under two light regimes: complete dark (24 h) and alternating light/dark (12/12 h), salinity levels in solutions of 0 (control), 50, 100, 200, 300, and 400 mM NaCl at 20/10°C and total darkness, osmotic stress at six osmotic potentials: 0 (control), -0.3, -0.6, -0.9, -1.2, and -1.5 MPa, pH conditions (4, 5, 6, 7, 8, and 9), and burial depths on the soil surface (0 cm) or buried at 1, 2, 3, 4, 5, and 6 cm of soil. The effect of mechanical scarification on germination was also evaluated.

Key results – Both species achieved high germination percentages (94–97%) at constant 21°C and alternating 20/10°C, with no significant differences between light and darkness. Germination decreased progressively with increasing salinity, remaining possible up to 300 mM NaCl for *O. caput-galli* and 400 mM for *O. conferta*. Both species tolerated osmotic stress down to -1.5 MPa, with *O. caput-galli* exhibiting greater tolerance. Germination occurred across a wide pH range (4–9), although acidic conditions reduced germination more strongly in *O. caput-galli*. Scarification significantly enhanced germination in *O. conferta*, while *O. caput-galli* germinated readily without treatment. Seedling emergence was observed from burial depths up to 5 cm, with optimal emergence at 1 cm.

Conclusion – The two species display broad ecological tolerance during germination and early establishment. Their threatened status is therefore more likely linked to in situ environmental pressures than to intrinsic germination constraints.

Keywords

dormancy, endangered endemic species, ex situ conservation, seed ecology

INTRODUCTION

Seed germination is a key stage in the plant life cycle, determining seedling establishment, population survival, and species distribution. Germination is affected by the interaction between seed dormancy characteristics and environmental conditions, including temperature, water availability, salinity, pH, light, and seed burial depth

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(Baskin and Baskin 1998). These environmental factors ensure that germination occurs when environmental conditions are suitable for seedling establishment and survival. Consequently, understanding germination ecology is fundamental for predicting species responses to climate change and for developing effective conservation, restoration, and propagation strategies (Hoyle et al. 2015; Frischie et al. 2020).

For rare and threatened species, germination ecology is particularly important because regeneration is often constrained by low seed viability, dormancy mechanisms, limited seedling establishment, and habitat degradation (Cousins et al. 2014; Arena et al. 2015; Miller et al. 2017). Understanding the germination ecology of these species facilitates the development of propagation protocols for ex situ conservation, ecological restoration, and population recovery. Germination studies have therefore become an essential component of conservation program, providing practical information for seed banking, habitat restoration, and reintroduction and restoration of threatened species (Griffiths et al. 2011; Commander et al. 2018; Frischie et al. 2020).

Among the environmental factors regulating germination, temperature and water availability are considered primary determinants of germination timing and success, while salinity, soil pH, light conditions, and burial depth further influence seedling emergence depending on species ecology (Brown et al. 2003; Hewitt et al. 2015; Espeland et al. 2017; Gioria et al. 2018). Mediterranean species are frequently exposed to seasonal drought, saline soils, and fluctuating temperatures, making germination responses to these abiotic factors particularly important for their persistence under current and future climate scenarios.

The genus *Onobrychis* Mill. (Fabaceae) comprises numerous perennial and annual legumes distributed mainly throughout Europe, western Asia, and the Mediterranean Basin, where many species are adapted to semiarid environments. In addition to their ecological importance, several *Onobrychis* species possess considerable agronomic value as forage legumes because of their drought tolerance, nitrogen-fixing capacity, and high nutritional quality. Wild relatives also represent valuable genetic resources for the development of climate-resilient forage crops in Mediterranean agriculture (Sakhraoui et al. 2023). In Tunisia, the genus is represented by four species: *O. conferta* (Desf.) Desv. subsp. *conferta*, *O. kabylica* (Bornm.) Širj., *O. caput-galli* Lam., and *O. crista-galli* Lam. (Pottier-Alapétite 1981; Le Houérou 1987; Abdelguerfi and Laouar 2002).

Among these taxa, *Onobrychis conferta* subsp. *conferta* is a rare perennial restricted to *Pinus halepensis* Mill. forests of Tunisia and Algeria. Due to severe habitat fragmentation, agricultural expansion, and overgrazing, the species has experienced a severe population decline and is currently classified as Critically Endangered according to IUCN criteria (Sakhraoui et al. 2024a). Likewise, *O. caput-galli* is a rare Mediterranean annual occurring in fragmented populations restricted to coarse sandy habitats, with recent surveys reporting new isolated populations in Tunisia (Pottier-Alapétite 1981; Sakhraoui et al. 2024b). The restricted distribution and conservation status of these species highlight the urgent need for effective strategies supporting their long-term preservation.

Among the Tunisian *Onobrychis* taxa, the studied species were selected because of their rarity, conservation relevance, and limited knowledge regarding their seed germination ecology. Their contrasting life histories also provide an opportunity to examine species-specific germination responses to Mediterranean environmental stresses.

Although seed germination has been investigated in several *Onobrychis* species, existing studies remain limited to a few cultivated or widespread taxa. Previous research has shown that *O. viciifolia* Scop. exhibits non-deep physiological dormancy (Baskin and Baskin 1998), while germination of *O. viciifolia* and *O. humilis* (Loefl.) G.López subsp. *humilis* is strongly influenced by temperature and, in some cases, requires cold stratification to overcome dormancy (Perez-Garcia et al. 1995). Moreover, interactions between temperature and soil moisture substantially affect germination performance and field emergence in *Onobrychis* species (Ehrman and Cocks 1996). Therefore, studies on additional wild species are necessary to identify species-specific germination strategies, particularly for rare taxa whose regeneration requirements remain poorly understood. These wild legumes represent potential reservoirs of adaptive traits, including tolerance to drought and environmental stress, which may contribute to future sustainable forage systems under climate change.

Despite their ecological and conservation significance, fundamental information on the germination ecology of *O. conferta* subsp. *conferta* and *O. caput-galli* is largely lacking. In particular, no comprehensive study has evaluated how multiple environmental factors interact to influence germination in these rare Mediterranean species. The absence of such information limits the development of efficient protocols for seed propagation, ex situ conservation, habitat restoration, and sustainable utilization. Furthermore, understanding their germination responses is increasingly important under projected climate change, which is expected to intensify drought and soil salinization throughout Mediterranean ecosystems (Macheroum and Chenchouni 2022).

To address this knowledge gap, the present study investigated the germination ecology of *O. conferta* subsp. *conferta* and *O. caput-galli* under controlled conditions. We evaluated the effects of pod removal, mechanical scarification, light regime, constant and alternating temperatures, salinity, osmotic stress, pH, and seed burial depth on germination. We hypothesized that pod removal and scarification would enhance germination by alleviating mechanical constraints imposed by the pod and seed coat. Given its occurrence in semiarid alkaline

habitats, we further expected *O. conferta* subsp. *conferta* to exhibit greater tolerance to salinity and alkaline conditions than *O. caput-galli*.

Through a comprehensive assessment of the germination requirements for these two rare Tunisian *Onobrychis* species, this study provides new insights into their regeneration ecology and provides practical information including ex situ propagation and potential in situ population reinforcement programs of wild genetic resources under Mediterranean environmental conditions.

MATERIAL AND METHODS

Seed collection

Mature dispersal units (pods) of *O. conferta* subsp. *conferta* were collected during their natural dispersal period in July 2018 from a roadside and the edge of cultivated land in Dyr Kef, north-western Tunisia (36°12'35.55"N, 8°44'32.90"E; 930 m a.s.l.). The collection area covered approximately 22 m^2 and included more than 30 mature plants to obtain a representative sample of the local population. The site is characterized by a Mediterranean climate, based on 25-year climatic records, with minimum and maximum annual air temperatures of 3.7°C and 35.1°C, respectively, and an average annual precipitation of 428.7 mm. These data provide a general description of the long-term climatic conditions of the habitat. The soil was classified as clay loam with low organic matter content (1.3%), high total limestone content (58%), and an alkaline pH of 7.8 (Sakhraoui et al. 2024a).

Similarly, mature pods of *O. caput-galli* were collected in July 2018 from a roadside population near Makthar in north-western Tunisia (35°54'7.83"N, 9°12'29.70"E; 839 m a.s.l.). The collection area extended across approximately 1000 m^2 and comprised more than 100 mature individuals to ensure broad genetic representation. Climatic conditions at the site included minimum and maximum annual temperatures of 4.9°C and 36°C, respectively, and annual rainfall averaging 384.6 mm. The soil was similar in texture and composition to that of *O. conferta*, being clay loam with 2.7% organic matter, 50% total limestone, and an alkaline pH of 7.2.

After harvest, pods of both species were manually cleaned by removing soil particles and plant debris. After cleaning, pods were stored in darkness at 4°C in a refrigerator under ambient relative humidity conditions (40–60%) for 14 days before the germination tests. Prior to experimentation, seeds were hand-separated from the pods, and only visually mature, undamaged seeds were selected. The mature pods of *O. conferta* subsp. *conferta* and *O. caput-galli* are indehiscent and contain a single seed. Seeds are small, reniform to ovoid, with a hard seed coat typical of Fabaceae species.

General germination procedure

Pods and seeds were sterilized in 5% (v/v) sodium hypochlorite for 15 s and thoroughly washed for 2 min with deionized water to minimize microbial contamination during incubation. To assess the effect of pod and seed structures on germination, intact pods, extracted seeds, and scarified seeds were placed on plastic Petri dishes (ten intact pods or ten individual seeds per dish) containing two layers of Whatman No. 1 filter paper, moistened initially with 5 mL of distilled water (controls) or the assigned test solution, and incubated in a germination chamber (Köttermann 2771, Uetze-Hänigsen, Germany) with fluorescent lamps that produced a photosynthetic photon flux density (PPFD) of 40 µmol photons $m^{-2}.s^{-1}$ (measured with an SB quantum 190 sensor, LI-COR Inc., Lincoln, NE, USA) and 12 h photoperiod. Petri dishes were enclosed and sealed with adhesive tape (Parafilm™) to prevent moisture loss. Each assay was conducted using a completely randomized design (CRD), where Petri dishes corresponding to different treatments were randomly distributed within the germination chamber. Each treatment consisted of ten replicates, with one Petri dish containing ten propagules (pods, seeds, or scarified seeds) per replicate. For the burial depth experiment, each replicate consisted of one pot containing 25 seeds. Seed germination was evaluated 14 days after the start of the experiment, after three consecutive days without further germination (ISTA 2018). Seeds were considered germinated when the radicle attained 2 mm length and seedlings emerged when cotyledons could be visually discerned. In all tests not involving temperature and light treatments, samples were incubated at the optimum condition for germination (20/10°C in the dark) recorded in the initial experiment on the effects of temperature and light. Seeds were transferred to new filter paper (Whatman No. 1) with new NaCl, PEG, and pH solution every 48 h to ensure relatively constant water potential in the treatments. Before the germination experiments, the average seed mass was determined using 200 randomly selected seeds weighed with an electronic balance (accuracy 0.01 g). This measurement was performed only for seed characterization and was not used as a criterion for seed selection.

Seed pod removal and scarification under different light and temperature regimes

To evaluate the influence of the pod and seed coat on germination, three propagule types were compared: (i) intact pods containing seeds, (ii) seeds manually removed from pods, and (iii) mechanically scarified seeds. Each propagule type was incubated under four alternating day/night temperatures (15/5, 20/10, 25/15, and 30/20°C) and four constant temperatures (17, 21, 25, and 29°C), under either continuous darkness or a 12 h light/12 h dark photoperiod. Pod removal was performed manually by carefully opening the dry pods with forceps and removing the enclosed seeds without damaging the seed

coat (Maldonado-Arciniegas et al. 2018). Temperature regimes were selected to encompass the range of day/night air temperatures recorded during the growing season at the collection site, based on 25-year climatic records. Darkness was achieved by wrapping Petri dishes in two layers of aluminium foil. Subsequently, Petri dishes in darkness treatments were opened under a green light to avoid any exposure to white light.

Effects of salinity, osmotic potential, and pH on germination

The effects of salinity on germination were determined by incubating seeds in solutions of 0 (control), 50, 100, 200, 300, and 400 mM NaCl at 20/10°C and total darkness.

The effects of osmotic potential on germination were determined by incubating seeds at six osmotic potentials: 0 (control), -0.3, -0.6, -0.9, -1.2, and -1.5 MPa adjusted by polyethylene glycol (PEG-6000, Loba Chemie Pvt. Ltd, Mumbai, India) to represent the range of soil water stress expected during the germination season in the natural habitats of *O. conferta* subsp. *conferta* and *O. caput-galli*, based on long-term environmental data reported by Nouiri (2024). Osmotic stress was developed by dissolving the required amount of PEG in distilled water according to the equation described by (Michel and Kaufmann 1973). The selected range of osmotic potentials simulated seasonal water stress levels in *O. conferta* and *O. caput-galli* habitats (Nouiri 2024).

The effects of pH on seed germination were determined by incubating seeds in six buffer solutions of pH 4, 5, 6, 7, 8, and 9. A 2-mM solution of MES (2-[N-morpholino] ethanesulfonic acid) was adjusted to pH 4, 5, or 6 with 1 N HCl or NaOH. A 2-mM solution of HEPES (N-[2-hydroxymethyl] piperazine-N′- [2-ethanesulfonic acid]) was adjusted to pH 7 or 8 with 1 N NaOH. A pH 9 buffer was prepared with 2-mM tricine (N-tris [hydroxymethyl] methyl glycine) and adjusted with 1 N NaOH. The selected pH range (4–9) encompassed both acidic and alkaline conditions to evaluate the tolerance of seed germination across a broad pH gradient, including and extending beyond the soil conditions typically encountered in the natural habitats of the two species.

To evaluate the reversibility of stress effects, ungerminated seeds remaining after 14 days of NaCl, PEG, and pH treatments were rinsed five times with bi-distilled water and transferred to Petri dishes containing two layers of filter paper moistened with 5 mL of distilled water. Seeds were then incubated for an additional 14 days under the same temperature conditions to assess recovery of germination after stress removal.

Effects of seed burial depth on germination and seedling emergence

The effects of seed burial depth on germination and seedling emergence were determined by placing 25 seeds on the soil surface (0 cm) or buried at 1, 2, 3, 4, 5, and 6 cm of soil within 30 cm diameter pots. This range was selected to represent shallow burial conditions expected in Mediterranean habitats and to determine the maximum depth allowing successful emergence. The soil used in the experiment was collected from the seed collection site (Dyr Kef, north-western Tunisia) to provide a substrate representative of the local habitat conditions and it was sieved through a 2 mm sieve before conducting the experiment. This experiment was designed to assess the influence of burial depth on emergence capacity rather than to replicate the complexity of natural soil seed banks. Additional pots containing the same soil but without added seeds were used as controls to verify the absence of viable background seeds in the collected soil. Pots were placed in the incubator set at the optimum condition for germination in the initial experiment on temperature and light and sub-irrigated with freshwater to avoid salinity effects. The number of emerged seedlings was counted daily for 21 days and expressed as the percentage of total seeds used.

Data analysis

At the end of germination experiments, the final germination percentage (FGP) was calculated as:

$$FGP\left(\%\right) = \frac{Nt}{N} \times 100$$

Where *Nt* is the number of seeds germinated in the respective treatments and *N* is the total number of seeds used in the bioassay.

Recovery percentage (RP) was calculated by the following formula:

$$RP = \frac{(a-b)}{(c-b)} \times 100$$

where *a* is the number of seeds germinated at 20/10°C and total darkness after a 14-day period plus those that recovered to germination at 21°C after another 14-day period, *b* is the number of seeds germinated under NaCl, PEG, and pH, and *c* is the total number of seeds tested.

Germination was monitored daily throughout the 14-day incubation period, and mean germination time (MGT) was calculated according to Ellis and Roberts (1981):

$$MGT = \frac{\sum Dn}{\sum n}$$

Where *n* is the number of seeds that had germinated on day *D* and *Dn* is the number of days counted from the beginning of germination experiment.

Statistical analyses were carried out using IBM SPSS Statistics for Windows (v.25.0, IBM Corp., Armonk, NY, USA), applying a significance level (α) of 0.05. Deviations from the arithmetic mean were calculated as standard error (SE). Normality and homogeneity of variance of data series were tested using the Kolmogorov–Smirnov and the Levene tests, respectively. General linear models (GLM/ANOVA) were used to compare FGP and MGT between salinity and osmotic treatments, and the percentage of viable seeds between burial depths. When

normality or homogeneity of variance was not achieved after transformations (using $\sqrt{x}$, 1/x, or ln(x) functions), data series were analysed using generalized linear models (GLZs) with a normal (Gaussian) distribution, and significance was assessed using the Wald χ^2 test (Ng and Cribbie 2017). When significant differences were found for a given plant trait, data were subjected to analysis of variance using a non-parametric Kruskal–Wallis H-test (Steel et al. 1997). Data of FGP and the percentage of seedling emergence were fitted to the best non-linear regression model in their response to salinity, osmotic potential, pH and soil burial depth using SigmaPlot for Windows (v.16.0, Systat Software Inc., San Jose, California, USA).

Artificial Intelligence use

During the preparation of this manuscript, the authors used Claude (Anthropic) to improve the clarity, language and readability of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

RESULTS

Effects of seed pod removal and scarification on germination under different light and temperature regimes

Seed germination of both *O. conferta* and *O. caput-galli* was significantly influenced by propagule treatments, light regimes, temperatures, and their interactions (all $p < 0.05$). In *O. conferta*, removing the pod significantly increased FGP by approximately 20% compared with intact pods, indicating that the pod structure partially restricted germination. Mechanical scarification had a stronger effect, increasing FGP by approximately 79% compared with untreated seeds, suggesting that the seed coat represented a major barrier to germination. In non-scarified seeds, germination was generally lower under alternating light/dark conditions than in darkness across most temperature treatments. This difference was reduced after seed scarification, indicating that the seed coat contributed to light sensitivity. However, light effects were not observed at the highest temperature treatments (29°C and 30/20°C). The highest FGP (97 ± 3%) was recorded in

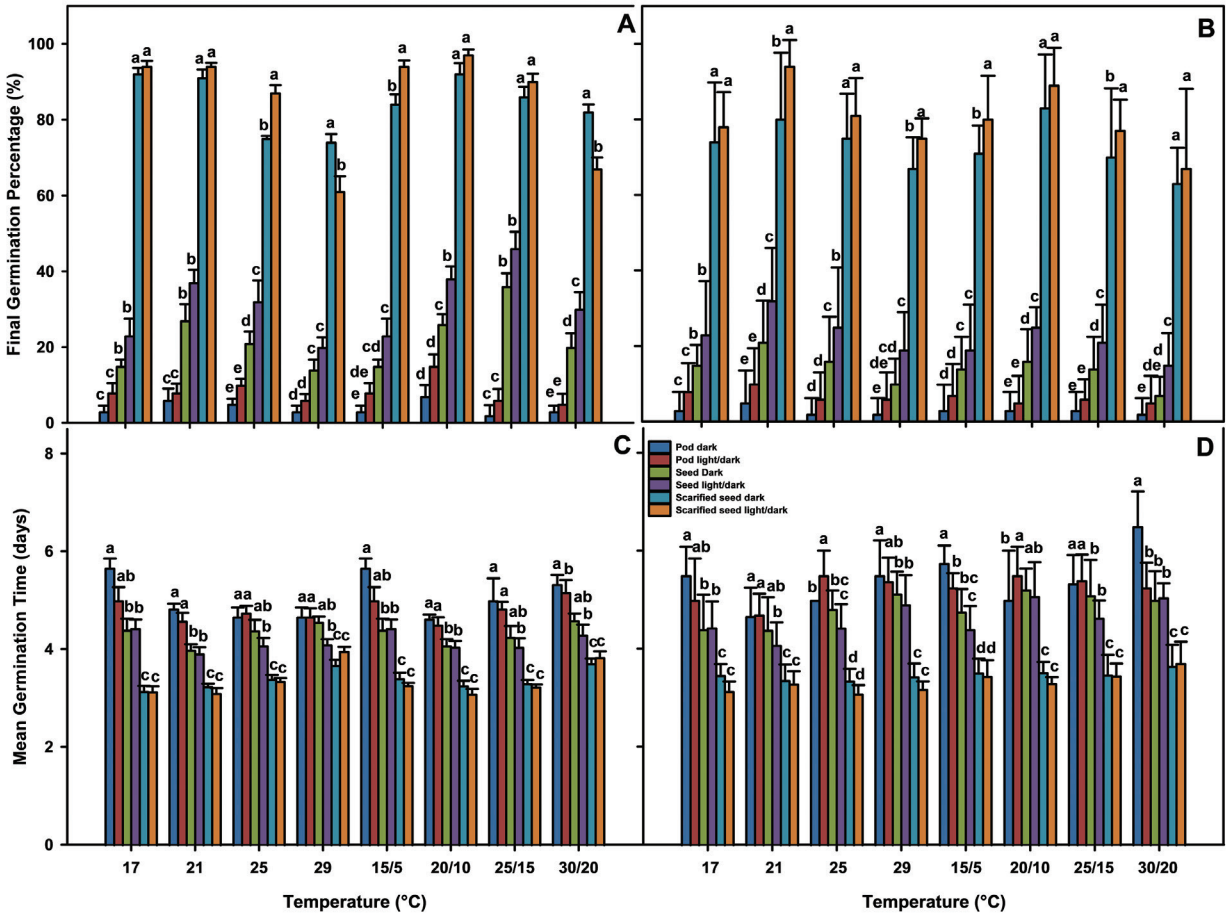

Figure 1. Effect of intact pods, seeds without pod, and scarified seeds incubated at light/dark and dark conditions at 17, 21, 25, 29, 15/5, 20/10, 25/15, and 30/20°C on final germination percentage (**A**, **B**) and mean germination time (**C**, **D**) of *Onobrychis conferta* subsp. *conferta* (A, C) and *O. caput-galli* (B, D). Values are mean ± SE (n = 10). Different letters indicate significant differences between treatments.

darkness at 20/10°C, while FGP decreased with increasing temperature (Fig. 1A, B).

Mean germination time was reduced by mechanical scarification, with the shortest MGT (3.1 days) recorded for scarified seeds at 20/10°C. In *O. caput-galli*, removal of the pod and seed scarification also enhanced germination, indicating that external seed structures restricted germination. Light conditions significantly affected germination in *O. caput-galli*. FGP was consistently higher in darkness (ca 34%) than under alternating light/dark conditions (ca 27%) across temperature treatments, indicating a negative effect of light exposure on germination. Temperature strongly influenced germination. The highest FGP was observed at 21°C (94%), followed by 20/10°C (89%), whereas both higher and lower temperatures reduced germination. Under dark conditions, germination at 21°C was statistically similar to that recorded at 20/10°C. The lowest germination percentages were observed at 30/20°C (58% in darkness and 53% under light/dark conditions), suggesting reduced germination capacity under high-temperature conditions. As with *O. conferta*, scarification reduced MGT in *O. caput-galli*, with the shortest MGT (3.1 days) at 25°C and the longest (6.5 days) recorded for seeds in intact pods at 30°C and 15/5°C (Fig. 1C, D).

Effects of salinity on germination

Salinity significantly affected both FGP and MGT in *O. conferta* and *O. caput-galli*, though their responses differed in pattern and tolerance thresholds ($p < 0.05$). In both species, germination followed a sigmoid response and exhibited an exponential decline in germination with increasing salinity, with the highest FGP (97 ± 3%) under control conditions (0 mM NaCl), maintaining FGP above 70% up to 200 mM NaCl. No germination was observed at 400 mM NaCl for *O. caput-galli* and nearly ceased (3 ± 0%) at 400 mM NaCl for *O. conferta* (Fig. 2A). In *O. conferta*, MGT increased significantly beyond 100 mM NaCl, from 3.1 ± 0.0 days under control to 6.5 ± 0.1 days at 400 mM. Similarly, in *O. caput-galli*, MGT rose from 3.2 ± 0.0 days at 0 mM to 5.3 ± 0.6 days at 300 mM NaCl (Fig. 2B). These results indicate that while both species are highly salt-tolerant, *O. conferta* shows slightly greater resilience to salinity stress than *O. caput-galli*.

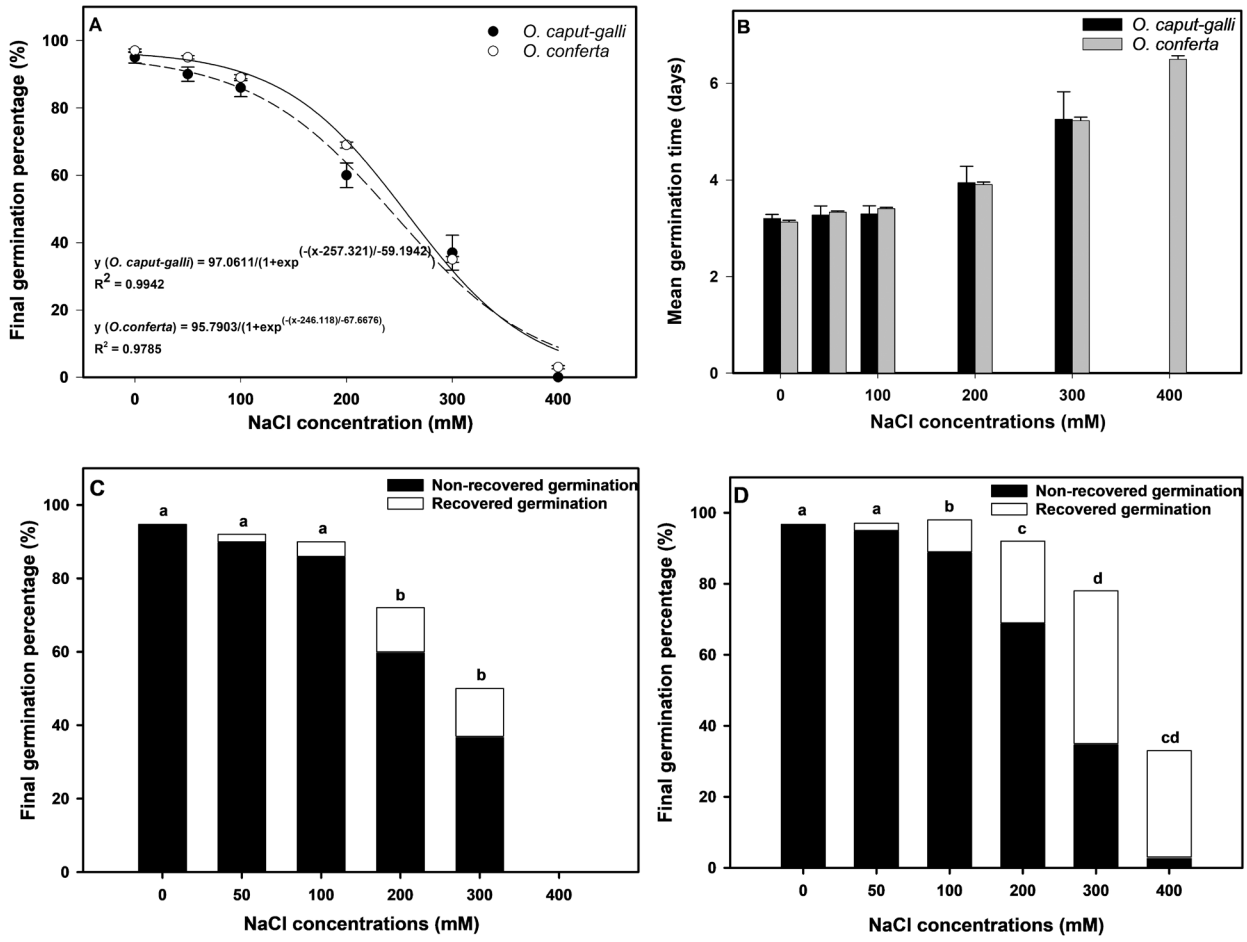

Figure 2. Effects of different NaCl concentration on final germination percentage (**A**), their corresponding mean germination time (**B**), and germination recovery of *O. caput-galli* (**C**) and *O. conferta* subsp. *conferta* (**D**) scarified seed at 20/10°C and total darkness. Values are mean ± SE (n = 10). Different letters indicate significant differences between NaCl concentrations (Kruskal–Wallis U test, $p < 0.05$).

A proportion of ungerminated, salt-treated seeds were able to germinate when they were transferred to distilled water for another 14 days (Fig. 2C, D). Recovery germination significantly ($p < 0.01$) increased with an increasing NaCl concentration. The highest RP values were reached for 300 mM NaCl (13% and 43% for *O. caput-galli* and *O. conferta*, respectively).

Effects of osmotic potential on germination

FGP and MGT in *O. conferta* and *O. caput-galli* were significantly influenced by water potential (osmotic stress) ($p < 0.05$). In *O. conferta*, FGP was highest (97 ± 1%) at 0.0 MPa and decreased markedly at water potentials below -0.9 MPa, reaching a minimum of 35 ± 1% at -1.5 MPa. Similarly, *O. caput-galli* exhibited maximum germination (94%) at 0 MPa, with a decrease to 46 ± 4% at -1.5 MPa. For both species, the osmotic potential causing a 50% reduction in germination was estimated at -1.4 MPa (Fig. 3A). Both species showed a significant increase in MGT with increasing osmotic stress, with the shortest germination times recorded at 0 MPa (3.2 ± 0.0 days) and the longest ca 5 days in *O. conferta* (between -1.2 and -1.5 MPa) and 4.9 ± 0.4 days in *O. caput-galli* at -1.5 MPa (Fig. 3B). These results demonstrate that osmotic stress reduces germination rates and delays germination timing in both species, with similar thresholds for 50% germination inhibition.

Seeds that did not germinate after 14 days of incubation in PEG solution were able to germinate when they were incubated for another 14 days in distilled water (Fig. 3C, D). There were a significant ($p < 0.05$) differences among recovery germinations for *O. conferta*. The RP value reached at -1.5 MPa (21%) was significantly ($p < 0.05$) different from all other RP values. No significant differences ($p > 0.05$) were observed among RP values for *O. caput-galli*, ranging from 3% to 6%.

Effects of pH on germination

Seed germination of *O. conferta* and *O. caput-galli* was significantly affected by pH, with both species showing optimal germination under neutral to slightly basic alkaline conditions ($p < 0.05$). In *O. conferta*, the highest FGP ranged from 97 ± 1% to 82% ± 2% at pH 6.6 to 9.0, while the lowest FGP (56 ± 1%) occurred at acidic

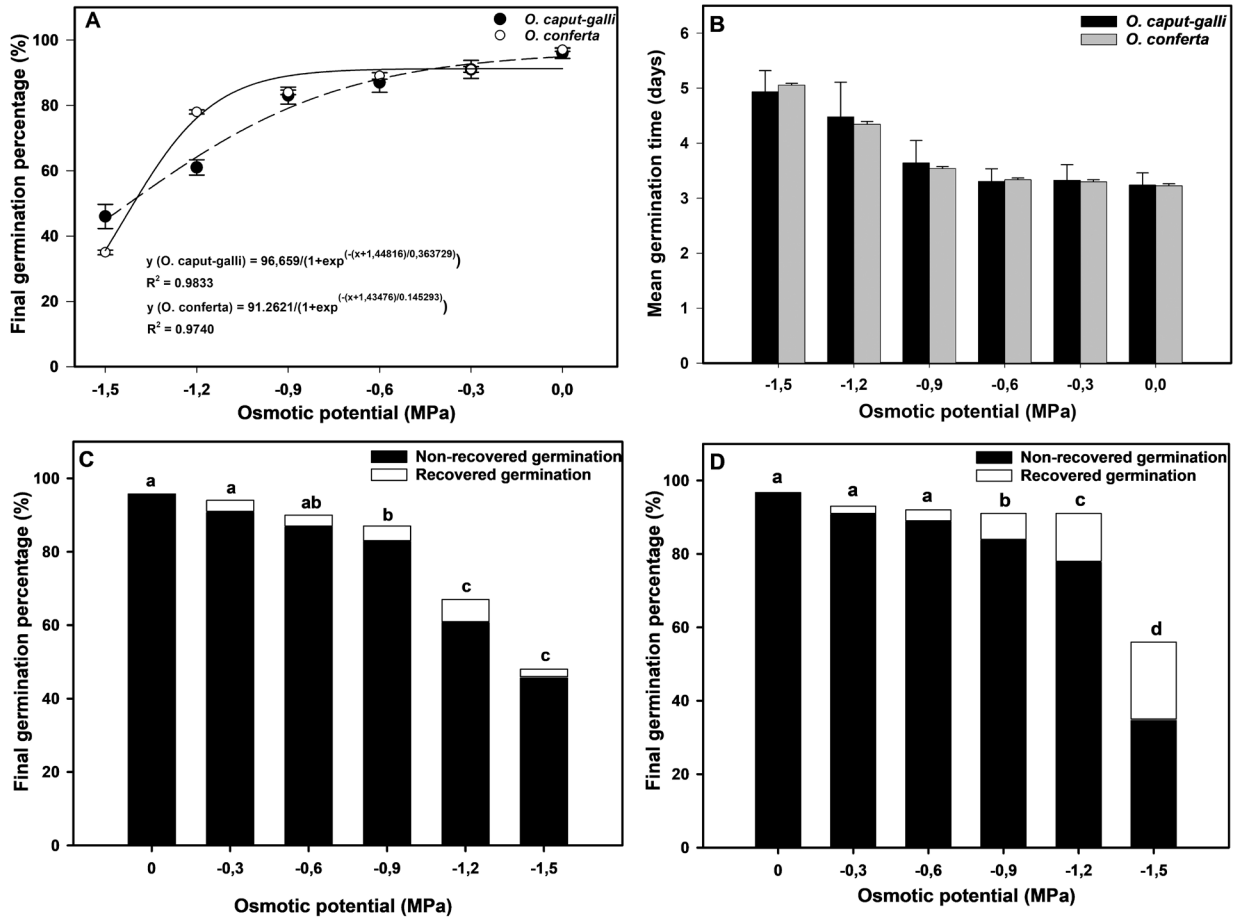

Figure 3. Effects of different osmotic potentials on final germination percentage (**A**), their corresponding mean germination (**B**), and germination recovery time of *O. caput-galli* (**C**) and *O. conferta* subsp. *conferta* (**D**) scarified seeds at 20/10°C and total darkness. Values are mean ± SE (n = 10). Different letters indicate significant differences between osmotic potentials concentrations (Kruskal–Wallis U test, $p < 0.05$).

pH 4 (Fig. 4A). The shortest MGT of 3.1 ± 0.0 days was recorded at pH 6.6, with delays observed at both low (4.1 ± 0.1 days at pH 4) and high pH (3.7 ± 0.1 days at pH 9.0). Similarly, *O. caput-galli* exhibited a quadratic response to pH, with germination decreasing from 92 ± 2% at neutral pH (7–8) to 47 ± 2% at pH 4. Both low (4.2 ± 0.4 days at pH 4) and high (3.9 ± 0.5 days at pH 9) pH levels delayed germination, and MGT peaked at pH values above 6.6 (Fig. 4B). These findings indicate that both species prefer neutral to slightly alkaline conditions for optimal germination, while extreme pH levels reduce germination success and slow germination rates.

A proportion of ungerminated, salt-treated seeds were able to germinate when they were transferred to distilled water (Fig. 4C, D). Germination recovery was significantly ($p < 0.01$) affected by pH level only for *O. conferta*. Ungerminated seeds under pH treatment showed recovery of germination, when transferred to distilled water, although lower recovery was observed for alkaline than acid environment (Fig. 4C, D). Recovery germination significantly ($p < 0.01$) decreased with a decreasing pH level. The highest RP values were recorded for a pH of 4 and 5 (15% and 10%, respectively) for *O. conferta* subsp. *conferta*.

Effects of seed burial depths on germination and seedling emergence

Seed germination, seedling emergence, and seed viability in *O. conferta* and *O. caput-galli* were significantly influenced by burial depth ($p < 0.05$). Both species showed germination at all depths tested; however, no seedlings emerged from seeds buried deeper than 5 cm in *O. conferta* and 6 cm in *O. caput-galli*. In *O. conferta*, the highest seedling emergence (92 ± 5%) occurred at 1 cm depth and declined to 36% at 5 cm, with a 50% inhibition of maximum emergence estimated at 4.6 cm. Similarly, *O. caput-galli* showed 80% emergence at the soil surface, decreasing exponentially with depth, and a 50% inhibition point at 4.2 cm (Fig. 5). These results indicate that while seeds can germinate at various depths, optimal seedling emergence occurs near the soil surface, with deeper burial negatively affecting emergence success in both species.

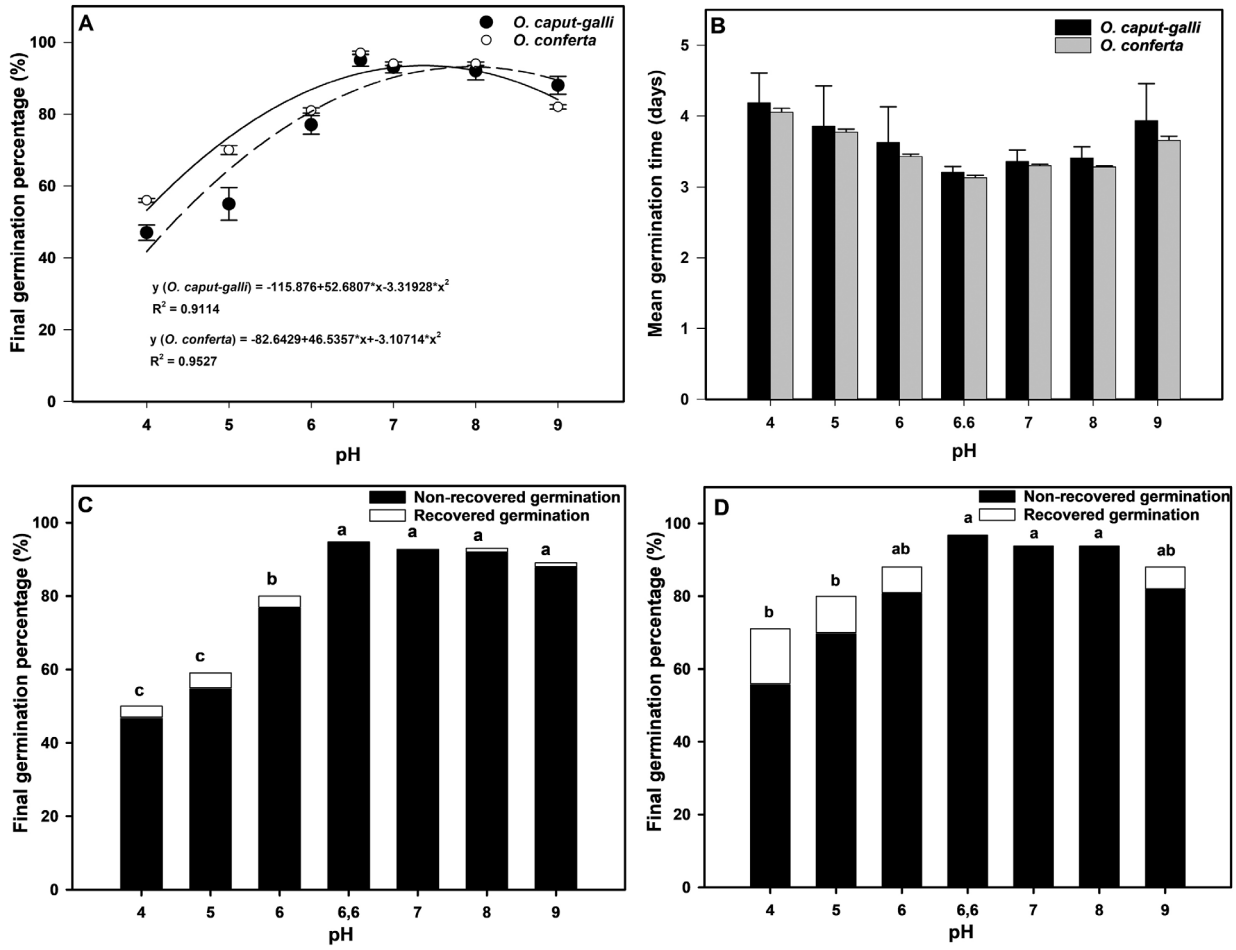

Figure 4. Effects of different pH levels on final germination percentage (**A**), their corresponding mean germination time (**B**), and germination recovery time of *O. caput-galli* (**C**) and *O. conferta* subsp. *conferta* (**D**) scarified seeds at 20/10°C and total darkness. Values are mean ± SE (n = 10). Different letters indicate significant differences between pH levels (Kruskal–Wallis U test, $p < 0.05$).

DISCUSSION

Our study reveals species-specific germination responses of *O. conferta* and *O. caput-galli* to multiple environmental factors, including pod removal, scarification, light, temperature, salinity, water potential, pH, and burial depth. These factors interact to regulate germination success and seedling emergence, influencing the recruitment strategies of both species in Mediterranean environments.

Seed germination in *O. conferta* and *O. caput-galli* is regulated by both chemical and physical constraints. The presence of physical dormancy imposed by the pod and hard seed coat represents an important mechanism controlling germination timing, as demonstrated by the enhanced germination following pod removal and scarification. Similar dormancy mechanisms have been reported in other Fabaceae, such as *Medicago truncatula* Gaertn. and *Trifolium pratense* L., where impermeable seed coats regulate the timing of germination as an adaptive strategy (Wang and Grusak 2005). Such dormancy allows seeds to remain viable until environmental conditions become favourable for successful seedling establishment (Taylor 2005; Chauhan et al. 2006).

Despite their contrasting life histories, *O. conferta* and *O. caput-galli* showed similar germination strategies, particularly the presence of physical dormancy and the ability to tolerate moderate environmental stress. These shared traits suggest common adaptive responses to seasonal drought and environmental variability in Mediterranean ecosystems. Similar dormancy release patterns have been described in other legumes, including *Lotus corniculatus* L. and related species, where seed coat impermeability regulates recruitment timing (Taylor 2005; Chauhan et al. 2006). In *O. conferta*, the broad tolerance to temperature, salinity, osmotic stress, and alkaline conditions is consistent with its occurrence in semiarid habitats characterized by variable water availability and alkaline soils (Muscolo et al. 2007; Wala et al. 2022). Such tolerance appears greater than that reported for several cultivated legumes, including *O. viciifolia*, *Pisum sativum* L., and *Phaseolus vulgaris* L., which are generally more sensitive to salinity and water stress during germination (Zarai et al. 2022). This physiological flexibility may contribute to the persistence of *O. conferta* in stressful Mediterranean habitats (Beyaz et al. 2011; Sakhraoui et al. 2023).

Mediterranean seasonal conditions is characterized by fluctuations in light availability and moderate abiotic stress. The preference for darker conditions suggests that germination may be favoured when seeds are partially covered by soil, where moisture conservation is greater and exposure to stressful surface conditions is reduced. Although its stress tolerance was lower than that of *O. conferta*, the germination response of *O. caput-galli* remains consistent with the environmental conditions of semi-arid Mediterranean habitats and with patterns reported for other wild *Onobrychis* species (Karamian and Ataei-Barazande 2013; Topçu and Özkan 2016; Uzun et al. 2017; Abbas et al. 2020).

The response of both species to burial depth highlights the importance of recruitment conditions after seed dispersal. Reduced emergence at greater depths likely reflects the limited capacity of seedlings to reach the soil

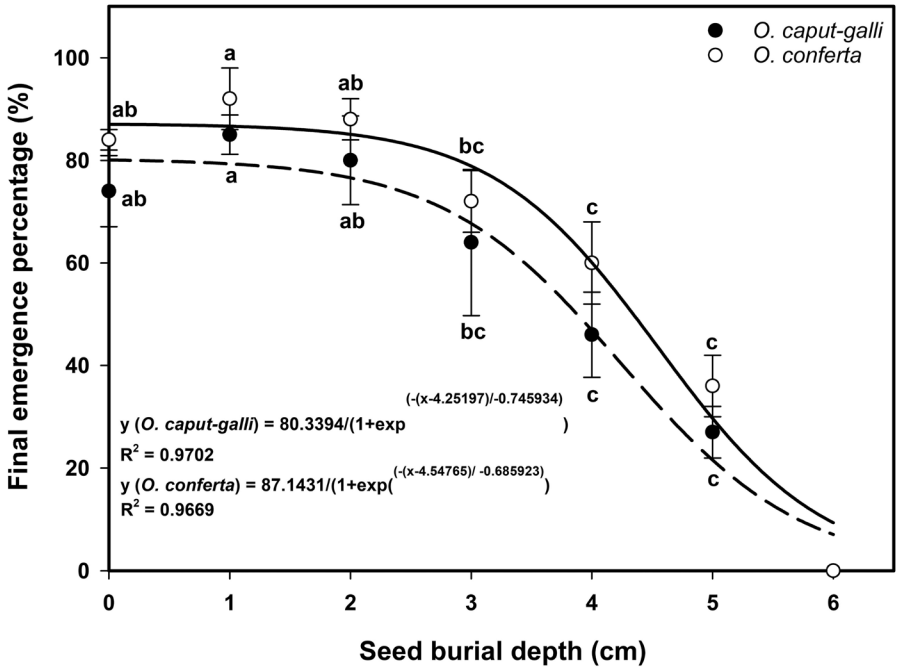

Figure 5. Effects of different seed burial depths on final emergence percentage of *O. conferta* subsp. *conferta* and *O. caput-galli* at 20/10°C and alternating light/dark (12/12 h). Values are mean ± SE (n = 10). Different letters indicate significant differences between seed burial depths (Kruskal–Wallis U test, $p < 0.05$).

surface when energy reserves are insufficient or when soil resistance restricts emergence. Similar depth-dependent emergence patterns have been reported in other Fabaceae, including *Stylosanthes hamata* Taub., where deeper burial strongly reduced seedling establishment (Lesturgez et al. 2004). The persistence of viable but ungerminated seeds under unfavourable conditions may represent a mechanism that spreads recruitment over time, reducing the risk of complete reproductive failure during unfavourable seasons. Such temporal spreading of germination is considered a bet-hedging strategy commonly observed in variable environments (Castillo et al. 2021).

The relatively rapid germination observed in *O. conferta* may represent an adaptive advantage in environments where favourable moisture conditions are short-lived. Similar rapid establishment strategies have been reported in stress-adapted Fabaceae such as *Cicer judaicum* Boiss. (Hanif et al. 2017; Castillo et al. 2022). Moreover, the ability of both species to germinate under reduced light availability may facilitate recruitment beneath vegetation cover or under shallow soil burial, where moisture retention is improved and temperature fluctuations are moderated (Bowers 2000; Cuena-Lombraña et al. 2020). These characteristics may contribute to successful establishment under the variable climatic conditions of Mediterranean ecosystems (Sakhraoui et al. 2024a).

The recovery of germination after removal of salinity, osmotic, and pH stresses indicates that these environmental constraints primarily induced reversible inhibition rather than permanent loss of viability. This response suggests that seeds can delay germination until stress conditions become less restrictive, representing an adaptive mechanism for environments characterized by unpredictable water availability. Similar conditional responses have been reported in stress-adapted Fabaceae, including *Medicago sativa* L. and *Lotus tenuis* Waldst. & Kit. ex Willd. The higher recovery capacity observed in *O. conferta* suggests greater physiological flexibility under fluctuating stress conditions (Rasheed et al. 2022).

The present study provides a comprehensive characterization of the germination ecology of *O. conferta* subsp. *conferta* and *O. caput-galli*. Our findings demonstrate that germination is regulated by interactions between seed structural traits and environmental conditions, including temperature, light, water availability, salinity, pH, and burial depth. These results improve understanding of the ecological requirements underlying seed recruitment in these poorly studied Mediterranean legumes and provide a foundation for future investigations under natural field conditions to assess seedling establishment, recruitment dynamics, and population persistence.

CONCLUSION

The present study provides the first comprehensive characterization of the germination ecology of *Onobrychis conferta* subsp. *conferta* and *O. caput-galli*. Germination in both species was strongly influenced by seed treatment, temperature, light, salinity, osmotic potential, pH, and burial depth. Pod removal and mechanical scarification markedly enhanced germination, indicating that physical barriers imposed by the pod and seed coat play an important role in regulating germination. Both species germinated optimally under moderate temperatures, in darkness, and under neutral to slightly alkaline pH conditions. Increasing salinity, osmotic stress, and burial depth progressively reduced final germination percentage and delayed germination, although many seeds remained viable and resumed germination after transfer from saline or osmotic stress conditions to distilled water. Seedling emergence was greatest at shallow burial depths and declined markedly with increasing soil depth.

Overall, the two species exhibited similar germination responses to the environmental factors investigated, despite differences in their life-history traits. These findings improve our understanding of the environmental conditions regulating germination in these poorly studied Mediterranean legumes and provide a foundation for future studies investigating seedling establishment, field recruitment, and population dynamics under natural conditions.

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